

1. *What is an **ångstrom unit**, and why is it used to describe molecular structures?*

The **ångstrom unit** is a unit of distance suitable for measuring atomic scale objects. 1 ångstrom (Å) = 1×10^{-10} m. The diameter of H atoms is just less than 1 Å, C is 1.54 Å, and the C-H bond is about 1 Å. Protein molecules have diameters of 20-100 Å

How does the ångstrom unit compare with the wavelength of light?

Visible light has wavelengths of 400-700 nm, or 4000-7000 Å. The smallest object that can be "seen" in the conventional sense must be larger than ½ the light wavelength, or 200 nm for blue light. Protein molecules are much too small to see by visible light.

Could we "see" a single protein molecule such as myoglobin?

It is possible to "see" a molecule if it emits its own light. This can be done by bonding a fluorescent dye onto a protein molecule. Fluorescence occurs when a molecule absorbs a quantum of light at one wavelength, and re-emits the energy as a quantum at a longer wavelength. However a fluorescent protein appears as a point of light, and no structural detail less than ½ wavelength of light can be resolved. Thus it's possible to use fluorescence to locate a protein in a cell, but not to see details of its structure.

Protein structures are determined by X-ray diffraction from crystals of the protein. The crystal contains a repeating array of protein molecules, and X-rays are deflected in characteristic patterns determined by the protein's structure. It is possible to reconstruct a model of the protein structure by mathematical analysis of the diffraction pattern. One can then "see" the structure by displaying the model on a computer.

It is also possible to "see" a protein molecule by using the electron microscope. The effective resolution of the electron microscope is of the order of 10-20 Å, so a protein will appear as an indistinct blob with no visible atomic structure. One of the problems is that the electron beam is very energetic and easily destroys structures made of C-C bonds.

2. *What are the characteristics of **alpha-helix**? of **beta-sheet**?*

A peptide chain is a chain of amino acids connected by peptide bonds. The atoms making up the peptide bond CO-NH are arranged in a rigid plane, due to the fact that the peptide bond behaves like a double bond. The α -C atom of each amino acid lies between the peptide bonds, and since this C is tetrahedral, it imposes a bend in the chain at each α -C.

Helix forms from a polypeptide chain in which the α -C atoms are **oriented to bend consistently in one direction**. The chain wraps around as a helix, the right-handed version of the helix being more stable than the left-handed. The helix can be wrapped more or less tightly, but the most stable structure (in terms of how atoms pack together) occurs when C=O of amino acid i lines up with NH of amino acid $i + 4$, allowing H-bonds to form. This is the α -helix, with 3.6 amino acids per turn of helix, with a period of 5.4 Å per turn of helix.

If the α -C bends in each amino acid alternate in orientation, the chain forms an **extended strand**. Amino acids occur every 3.5 Å in opposite orientations along the extended strand, and every 7.0 Å in equivalent orientation.

No H-bonds form within a single extended strand, but two or more extended strands can line up side by side. If the strand directions are opposed, this aligns NH groups on one strand with C=O groups on the other, allowing good interstrand H-bonds to form. This is the antiparallel β -sheet. If the strand directions are the same H-bonds form, but are not as well aligned. This is the parallel β -sheet.

Why do some amino acids prefer one type of secondary structure over the other?

The side chains of the amino acids lie up against the side of the α -helix, and serve to protect the core H-bonds from disruption by the surrounding H₂O. α -Helix can become crowded when side chains are bulky or branch on the β -C atom. Extended strands orient the side chain perpendicular to the strand axis, and give the maximum space for bulky amino acid side chains. Hence the bulky amino acids Tyr, Trp, Val, Ile, Thr, Cys prefer β -sheet over α -helix, and this structure tends to form when these amino acids are in majority. Otherwise the "default" structure is α -helix. Phe supports forming a secondary structure, but without particular preference for alpha or beta structure.

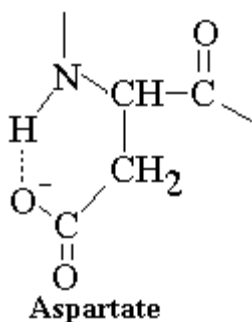
Why do some amino acids not favour secondary structure?

Some amino acids fail to protect the backbone H-bonds and may actively disrupt them. The sidechain of Gly is a single H atom. Unless neighbouring amino acids have large sidechains, the presence of Gly may leave a gap in the shielding side chains that allows H₂O. Gly is often found at the C-terminal end of an α -helix, where it allows H₂O in to terminate the chain of H-bonds that makes up the helix.

Pro has a side chain that bonds covalently to its own α -N atom to form a ring. Apart from the fact that the α -N lacks an H to donate as an H-bond, a -CH₂- group occupies the space where an incoming C=O might be positioned. The Pro C=O group can H-bond, so Pro can often be found at the N-terminal end of an α -helix where the C=O groups participate in H-bonds, but NH groups don't.

Ser, Asn and Asp have H-bonding side chains which are just the right length to H-bond to their own backbone groups. The "right size"

sidechain allows a 6-member ring to form which is relatively stable due to tetrahedral geometry of C-atoms. Glu would make 7-member ring which is quite awkward to form.



Instead of protecting secondary structure H-bonds, these sidechains actually disrupt them.

3. *What **non-covalent forces or interactions** help stabilize the folded tertiary structures adopted by polypeptides?*

The most important single controlling protein structure is the **hydrophobic interaction** that causes non-polar amino acids to gather in the core of the protein to minimize the area of contact with H₂O. Non-polar amino acids on or near the surface of a protein don't destabilize the structure, but they do create a "sticky patch" which could be used to help bind a ligand, or to stick two protein subunits together (such as the bonding between α1 and β1 or α2 and β2 in hemoglobin structure).

Shapes should be more or less complementary, since matched shapes result in more close atom to atom contacts and close atom to atom contacts maximize the van der Waals interaction. Ion pairs and side chain H-bonds also occur, but to a more limited extent in the folding of a monomeric protein like myoglobin, primarily because H-bonding and charged amino acids tend to be on the protein surface where they can interact with H₂O and ions in the surrounding solution. H-bonds and ion pairs play a bigger role in binding of ligands and binding of one subunit to another in a quaternary structure, since amino acids on the surface will be involved in protein-ligand or subunit-subunit interactions

*What is a **disulfide bond**, and why is it less common than once thought?*

Disulfide bonds form by oxidation of pairs of Cysteines which have their side chains -CH₂-SH in close proximity in the folded protein.



This creates a strong covalent bond helping hold a folded protein together. Among the first proteins to have their structure determined, insulin and ribonuclease both have several disulfide bonds, so this phenomenon found its way into the textbooks. However, disulfide formation requires oxidizing conditions, and conditions inside the cell are reducing. Now that many thousands of protein structures have been determined, it's been found that disulfide bonds are almost exclusively found on proteins that exist outside cells, where the disulfides give the protein extra stability that might be needed in the harsher environment outside cells.

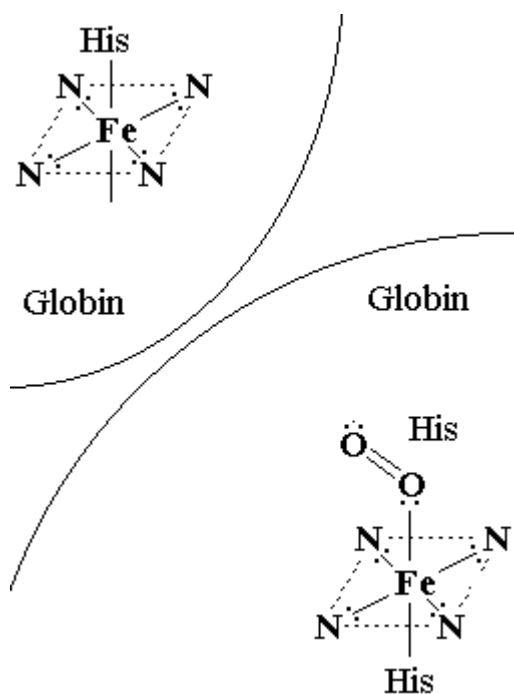
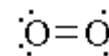
4. *Why is simple ionic **Fe²⁺** not used to transport O₂?*

Simple Fe²⁺ ions are prone to oxidation by bound O₂. They might bind O₂, but would release hydroxyl radical or peroxide anion. Once oxidized to Fe³⁺, the ion no longer binds O₂, but binds H₂O as ligand instead.

Heme provides a framework of 4 out of the 6 potential ligands for Fe²⁺, so it binds Fe²⁺, and sequesters the free ion from the solution phase within the cell.

Globin **protects** the heme, and provides some of the **selectivity so that O₂ is bound more favourably**. Globin provides ligand 5 (His F8), leaving position 6 free for some other ligand to bind. His E7 partly obstructs ligand 6, so that diatomic molecules like

:C≡O: which has one lone pair on each atom in a linear arrangement are bound less favourably, while O=O, which has two lone pairs on each O atom in a trigonal planar arrangement can bind better. Without this help, CO would bind 20000 time more tightly than O₂.



Why is heme-Fe(II) embedded in globin so as to function as an oxygen-storage or oxygen transporting molecule?

Oxidation of Fe²⁺ by O₂ proceeds by a mechanism where **one O₂ molecule is sandwiched between two Fe²⁺ ions**, both in the case of simple Fe²⁺ ions, or as heme Fe²⁺.

When heme is embedded in globin, the surrounding protein prevents heme-Fe²⁺ molecules from lining up with a second heme at the right distance to form the sandwich complex with O₂.

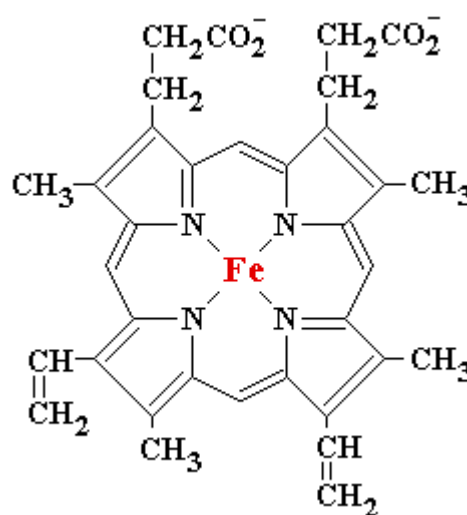
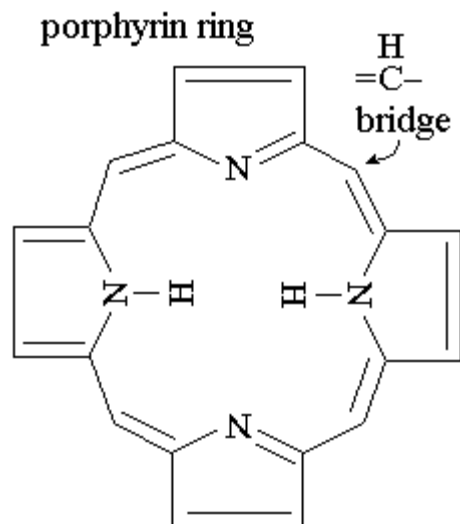
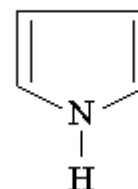
5. What is meant by the terms *pyrrole*, *porphyrin*, *protoporphyrin IX*?

Pyrrole is a simple 5 member ring with one N atom.

Porphyrin describes a family of compounds made up of **four pyrrole units** linked by CH bridges, which have **various side chains** added to the simple pyrrole structure.

Protoporphyrin IX has the particular porphyrin structure found in cytochromes, hemoglobin and myoglobin. Protoporphyrin IX + Fe²⁺ is heme.

Pyrrole ring



6. *Distinguish between the meanings of the term **ligand** in the context of **proteins** and **metal ions**.*

A ligand is simply a molecule that can be bound by another atom or molecule.

Any molecule can serve as a ligand for a protein, provided that the protein has a binding site that is complementary to it in terms of matching hydrophobic regions, overall shape, H-bonding groups and charged groups. In other words, it's the **structure of the protein that determines whether a particular ligand can bind or not**.

A ligand for a metal ion must be able to **donate a lone pair to the metal**, helping to occupy the metal ion's valence shell of electrons. For transition metals, the valence shell may extend into the d orbitals, so it's not uncommon to see transition metals accept 6 ligands.

7. *Amino acids in most proteins are identified by their position in the sequence, for example, Asp102, His 57 and Ser 195 in chymotrypsin. For globins, the helices that make up the tertiary structure are identified by letters **A-H**, where helix A is closest to the N-terminus. Amino acids then identified by position within that helix, e.g. **His E7**. What is the significance of **His E7** to globin function?*

Although the overall appearance of the tertiary structure of the globins is similar, there are variations in the sequences and in the length of some of the connecting loops. Taking a particular position in the overall sequence does necessarily mean the same location in the tertiary structure (See Lehninger p. 211). Amino acid 93 is the ligand 5 His in myoglobin, valine in Hb α and Cys in Hb β , none of which are in equivalent locations in the tertiary structure. However position F8, the 8th amino acid in helix F is always His, and is always ligand 5 for heme Fe²⁺.

Position E7 is also always His, in a position relative to heme known as the *distal His*, since it is too far away to serve as ligand 6. His E7 serves to strengthen the binding of O₂ relative to the binding of CO by heme Fe²⁺ by forcing diatomic ligands to bind at an angle. (See question 4 or Lehninger p.209 Fig. 7.5)

8. *Indicate yes or no to complete the following table:*

	Heme-Fe(II)	Myoglobin	Hemoglobin
Contains protein	no	yes	yes
Water soluble	yes	yes	yes
Binds O ₂	yes, but not reversibly	yes	yes
Reduced affinity for CO	no	yes	yes
Binds O ₂ cooperatively	no	no	yes

9. What would be the physiological effect if **hemoglobin** in red blood cells was replaced by **myoglobin**?

O₂ would be bound extremely tightly. Since $P_{50} = 0.26$ kPa for myoglobin and pO₂ is 12 kPa in lungs, myoglobin reaches 98% O₂ occupancy. However on reaching the tissues where normally pO₂ is about 4 kPa, occupancy will drop to about 94%, so only a small fraction of the O₂ carried can be released. Under these conditions one might expect the tissues to be O₂-depleted. Even if pO₂ drops to 1 kPa, O₂ occupancy will remain at ~80%, and only 18% would be released.

10. What are the **structural** differences between myoglobin and hemoglobin? What are the **similarities**? What are the **functional** differences between hemoglobin and myoglobin?

Myoglobin shares similar secondary and tertiary structure with the individual globin subunits of hemoglobin (Hb α actually lacks helix D, see Lehninger p.211, but the correspondence of the other 7 helices is consistent.). The amino acid sequences match well for amino acids that contact the heme directly.

Amino acids that appear on the surface of the globins vary most. Partly this is because there is less constraint on side chains that face the exterior, because they don't have to maintain such a close fit as side chains in the interior. However another factor is that myoglobin subunits are designed not to bind to other myoglobins, whereas different surfaces on Hb α and Hb β are designed to interact with each other. Helices G and H form contacts in the strong $\alpha 1-\beta 1$ and $\alpha 2-\beta 2$ interactions, with several polar amino acids in the myoglobin sequence replaced by nonpolar, e.g. Arg H17 in myoglobin becomes Ala in Hb β , and Glu H14 becomes Ala in both Hb α and Hb β .

Other amino acids are responsible for highly specific interactions between $\alpha 1-\beta 2$, $\alpha 1-\alpha 2$ and $\beta 1-\beta 2$ which are critical for controlling the switch between low affinity T-state and high affinity R-state of hemoglobin.

Functionally, myoglobin and hemoglobin are O₂ binding proteins, but whereas myoglobin has simple binding kinetics suitable for a storage protein, the way hemoglobin binds O₂ is optimized for its role as a transport protein. The ability to switch between high affinity state when O₂ is being loaded and low affinity state when O₂ is being released make for much more efficient transfer of O₂ from lungs to the body tissues.

Here's another question for you to think about:

Globins form highly stable $\alpha\beta$ dimers. Is it possible for the $\alpha\beta$ dimer to show allosteric effects? If so, how? If not, why not?

Answer will appear next week.